AMENDMENTS

In The Claims:

1. (Amended) A method for [high-throughput amplification of] obtaining host cells that identify the function of [one or more members of an oligonucleotide family which is related to] a product of a sample nucleic acid, said method comprising the steps of:

[growing] expressing one or more members of an oligonucleotide family in a high-throughput format as individual transcription products in a multiplicity of recombinant non-bacterial host [cell cultures] cells, wherein the coding sequence for said individual transcription products is contained in an expression vector lacking bacterial or bacteriophage cloning sequences and codes for an antisense nucleic acid which when expressed as RNA binds to a mRNA sequence transcribed from a target RNA sequence that comprises a nucleotide sequence of said sample nucleic acid, wherein expression of one or more of said individual transcription products prevents production of a product of said mRNA [containing an expression vector comprising at least one member of said oligonucleotide family] ; and [whereby said member is transcribed and the number of copies of said oligonucleotide is amplified in each of said non-bacterial cell cultures]

isolating host cells that have an altered phenotype.

2. (Amended) The method according to Claim 1, wherein a transcription product of said one or more members of said oligonucleotide family is a ribozyme [designed to cleave a target nucleic acid expressed by said non-bacterial cell cultures, wherein said target nucleic acid comprises a nucleotide sequence of said sample nucleic acid].

3. (Amended) The method according to Claim 1, wherein a transcription product of said one or more members of said oligonucleotide family is an antisense nucleic acid [designed to bind to a target nucleic acid expressed by said non-bacterial cell cultures, wherein said target nucleic acid comprises a nucleotide sequence of said sample nucleic acid].

4. (Amended) The method according to Claim 1, wherein said recombinant non-bacterial host [cell] cells [cultures] comprise mammalian cells.

5. (Reiterated) The method according to Claim 1, wherein said expression vector is a plasmid or a virus.

6. (Reiterated) The method according to Claim 5, wherein said virus is a retrovirus, or an adeno-associated virus.

7. (Amended) The method according to Claim 1, wherein said sample nucleic acid is a genomic DNA, a cDNA, an [essential] expressed sequence tag (EST), or an RNA.

8. (Amended) A method of assigning a function to a product coded for by a sample nucleotide sequence, said method comprising:

[growing a cell culture comprising one or more host cell(s) wherein said host cells express a target nucleic acid comprising said sample nucleotide sequence and wherein said host cells contain one or more members of a family of nucleic acids which bind to a transcription product of said nucleotide sequences whereby transcription product of said target nucleic acid is inhibited and said host cell exhibits at least one phenotypic change]

analyzing phenotypic changes in [said cell] host cells obtained according to the method of Claim 1 [to identify one or more altered function(s)] as indicative of the function of a product of said sample nucleic acid; and

obtaining a nucleotide sequence of said target nucleic acid, whereby a function is assigned to a product of said sample nucleotide [sequences] sequence.

9. (Reiterated) The method according to Claim 8, wherein said function is a physiological function.

10. (Reiterated) The method according to Claim 8, wherein said function is enzyme activity.

11. (Reiterated) The method according to Claim 8, wherein said function is protein synthesis.

12. (Reiterated) The method according to Claim 8, wherein said function is expression of a biological factor.

13. (Reiterated) The method according to Claim 8, wherein said function is a regulatory effector function.

14. (Amended) The method according to Claim 8, wherein said altered function is [altered] monitored directly.

15. (Amended) [A] An expression vector comprising:

a double-stranded DNA comprising[:] a sense strand and an antisense strand, wherein said sense strand codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a mammalian target nucleic acid sequence so that expression of a product of said mammalian target nucleic acid is inhibited, wherein a means for determining directionality of expression is included in said double-stranded DNA; wherein said product is associated with at least one phenotypic property of a host cell comprising said mRNA sequence; and wherein said expression vector lacks bacterial or bacteriophage cloning sequences.

16. (Reiterated) The [double-stranded DNA] expression vector according to Claim 15, wherein said RNA comprises a catalytic domain which when expressed as RNA cleaves a mRNA sequence transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA.

17. (Reiterated) The [double-stranded DNA] expression vector according to Claim 16, wherein said RNA is a ribozyme.

18. (Reiterated) The [double-stranded DNA] expression vector according to Claim 15, wherein said means for determining directionality of expression comprises a different non blunt-ended restriction enzyme site at each end of said double-stranded DNA.

19. (Reiterated) The [double-stranded DNA] expression vector according to Claim 18, wherein said double-stranded DNA is formed by contacting a first oligonucleotide with a complementary second oligonucleotide.

20. (Reiterated) The [double-stranded DNA] expression vector according to Claim 19, wherein said non blunt-ended restriction enzyme site is complementary to an end of said expression vector.

21. (Amended) [A] An [delivery] expression vector [comprising the double-stranded DNA] according to Claim 15, wherein said [delivery] expression vector is formed by contacting a double-stranded oligonucleotide with an expression vector.

22. (Amended) [A] An [delivery] expression vector [comprising the double-stranded DNA] according to Claim 15, wherein said [delivery] expression vector is formed by contacting a single-stranded oligonucleotide with [an] said expression vector.

23. (Amended) [A] An [delivery] expression vector [comprising the double-stranded DNA] according to Claim 15, wherein said double-stranded DNA is formed by contacting a triple-stranded oligonucleotide with an expression vector.

24. (Amended) [The] A triple-stranded oligonucleotide [according to Claim 23], wherein said triple-stranded oligonucleotide is [formed] by the method of contacting a first oligonucleotide, a second oligonucleotide and a third oligonucleotide, wherein said second oligonucleotide is complementary to nucleotides at the 5' end of said first oligonucleotide, wherein said second oligonucleotide further comprises excess nucleotides extending beyond the 5' end of said first oligonucleotide, wherein said third oligonucleotide is complementary to nucleotides at the 3' end of said first oligonucleotide, and wherein said third oligonucleotide contains excess nucleotides that extend beyond the 3' end of said first oligonucleotide.

25. (Reiterated) The triple-stranded oligonucleotide according to Claim 24, wherein said excess nucleotides are complementary to and base pair with the ends of said expression vector.

26. (Amended) The [double-stranded DNA] delivery vector according to Claim 22 or Claim 23, wherein said expression vector is filled in with Klenow.

27. (Amended) The [double-stranded DNA molecule] delivery vector according to Claim 21, Claim 22, or Claim 23, wherein said expression vector further comprises regulatory elements for expression.

28. (Amended) A retrovirus expression vector comprising:

[a retrovirus plasmid vector comprising] a double-stranded DNA [according to Claim 15] comprising a sense strand and an antisense strand, wherein said sense strand codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a target nucleic acid sequence so that expression of a product of said target nucleic acid is inhibited, wherein a means for determining directionality of expression is included in said double-stranded DNA , wherein said product is associated with at least one phenotypic property of a host cell comprising said mRNA sequence; and wherein said retrovirus expression vector lacks bacterial or bacteriophage cloning sequences.

29. (Reiterated) The retrovirus expression vector according to Claim 28, wherein said vector comprises supercoiled DNA.

30. (Reiterated) A retrovirus packaging cell line comprising:

a retrovirus expression vector according to Claim 28.

31. (Amended) A retrovirus particle comprising:

a genome encoding expression of an RNA comprising a catalytic domain for cleavage of a mRNA transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA , wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA.

32. (Amended) [A mammalian cell comprising:

one or more double-stranded DNA(s) comprising a sense strand and an antisense strand, wherein said sense strand codes for a catalytic domain which when expressed as RNA cleaves a mRNA sequence transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA, and wherein a means for determining directionality of expression is included in said one or more double-stranded DNA(s)] Host cells obtained according to the method of Claim 1, wherein said host cells are mammalian host cells.

33. (Amended) An adeno-associated virus expression vector comprising:

[an adeno-associated virus plasmid vector comprising] a double-stranded DNA [according to Claim 15] comprising a sense strand and an antisense strand, wherein said sense strand codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a target nucleic acid sequence so that expression of a product of said target nucleic acid is inhibited, wherein a means for determining directionality of expression is included in said double-stranded DNA , wherein said product is associated with at least one phenotypic property of a host cell comprising said mRNA sequence; and wherein said expression vector lacks bacterial or bacteriophage cloning sequences.

34. (Amended) An adeno-associated virus packaging cell line comprising:

an adeno-associated virus [plasmid] expression vector according to Claim 33 and an adeno-associated virus helper plasmid.

35. (Amended) An adeno-associated virus comprising:

a genome encoding the expression of an RNA comprising a catalytic domain for cleavage of a mRNA transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA , wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA.

36. (Reiterated) A plasmid expression vector comprising:

a double-stranded DNA [according to Claim 15] comprising a sense strand and an antisense strand, wherein said sense strand codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a target nucleic acid sequence so that

expression of a product of said target nucleic acid is inhibited, wherein a means for determining directionality of expression is included in said double-stranded DNA , wherein said product is associated with at least one phenotypic property of a host cell comprising said mRNA sequence; and wherein said expression vector lacks bacterial or bacteriophage cloning sequences.

37. (Amended) A method for the introduction into a host cell(s) of a plasmid expression vector, said method comprising:

contacting a cell culture comprising one or more host cell(s) with a co-precipitate of calcium phosphate and a double-stranded DNA comprising a sense strand and an antisense strand, wherein said sense strand codes for a catalytic domain, which when expressed as RNA cleaves a mRNA sequence transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA, [and] wherein a means for determining directionality of expression is included in said double-stranded DNA , and wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA, whereby said plasmid expression vector is introduced into said host cells.

38. (Reiterated) The method according to Claim 37, wherein said host cell(s) comprise mammalian cell(s).

39. (Reiterated) The method according to Claim 37, wherein said plasmid expression vector is supercoiled DNA.

40. (Amended) A method for expressing in a host cell a plasmid expression vector, said method comprising:

introducing into said host cell said plasmid expression vector comprising a double-stranded DNA comprising a sense strand and an antisense strand, wherein said sense strand codes for a catalytic domain, which when expressed as RNA cleaves a mRNA sequence transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA, [and] wherein a means for determining directionality of expression is included in said double-stranded DNA, and wherein said plasmid expression vector

lacks bacterial or bacteriophage cloning sequences, whereby said plasmid expression vector is expressed in said host cells.

Cancel Claim 41.

[41. The method according to Claim 40, wherein said plasmid expression vector is expressed in said host cell(s) without an intervening bacterial cloning step.]

[42] 41. (Renumbered) The method according to Claim 40, wherein said plasmid expression vector is a retrovirus expression vector.

[43] 42. (Renumbered) The method according to Claim 40, wherein said plasmid expression vector is an adeno-associated virus expression vector.

[44] 43. (Renumbered) The method according to Claim 40, wherein said plasmid expression vector is contacted with gyrase.

[45] 44. (Renumbered and Amended) A method for construction of a ribozyme vector, said method comprising:

inserting a double-stranded DNA into a linearized delivery vector, wherein said double-stranded DNA comprises a sense strand and an antisense strand, wherein said sense strand codes for a catalytic domain which when expressed as RNA cleaves a mRNA sequence transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA , wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA, and wherein said ribozyme vector lacks bacterial or bacteriophage cloning sequences.

[46] 45. (Renumbered and Amended) The method according to Claim [45] 44, wherein said double-stranded DNA is formed by contacting a first oligonucleotide with a complementary second oligonucleotide.

[47] 46. (Renumbered and Amended) The method according to Claim [46] 45, wherein a means for determining directionality of expression is included in said double-stranded DNA.

[48] 47. (Renumbered and Amended) The method according to Claim [47] 46, wherein said means for determining directionality of expression comprises a different non blunt-ended restriction enzyme site at each end of said double-stranded DNA.

[49] 48. (Renumbered and Amended) The method according to Claim [45] 44, wherein said double-stranded DNA is formed by annealing a first oligonucleotide, a second oligonucleotide, and a third oligonucleotide, wherein said second oligonucleotide is complementary to nucleotides at the 5' end of said first oligonucleotide, wherein said second oligonucleotide further comprises excess nucleotides extending beyond the 5' end of said first oligonucleotide, wherein said third oligonucleotide is complementary to nucleotides at the 3' end of said first oligonucleotide, and wherein said third oligonucleotide contains excess nucleotides that extend beyond the 3' end of said first oligonucleotide.

[50] 49. (Renumbered and Amended) The method according to Claim [49] 48, wherein said excess nucleotides are complementary to and base pair with the ends of said linearized delivery vector.

[51] 50. (Renumbered and Amended) The method according to Claim [49] 48, wherein said ribozyme vector is treated with a DNA polymerase.

[52] 51. (Renumbered and Amended) The method according to Claim [51] 50, wherein said DNA polymerase is a cellular DNA polymerase.

[53] 52. (Renumbered and Amended) The method according to Claim [51] 50, wherein said DNA polymerase is a Taq DNA polymerase.

[54] 53. (Renumbered and Amended) The method according to Claim [51] 50, wherein said DNA polymerase is Klenow.